PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Optimizing Mycobacterium tuberculosis detection in resource limited settings
AUTHORS	Alfred, Nwofor; Lovette, Lawson; Aliyu, Gambo; Obasanya, Joshua; Meshak, Panwal; Jilang, Tunkat; Iwakun, Mosunmola; Nnamdi, Emenyonu; Olubunmi, Onuoha; Dakum, Patrick; Abimiku, Alash'le

VERSION 1 - REVIEW

REVIEWER	Yap Boum II Epicentre Mbarara, Uganda
REVIEW RETURNED	18-Oct-2013

GENERAL COMMENTS	The paper written by Gambo et al evaluating different microscopy methods and digestion time for culture is quite relevant for RLS since the recommendation of WHO to rule out Fluorescence microscopy for TB diagnosis. however no ethical approval has been mentioned in the manuscript which is critical for the running of any study involving human subject. in the entire manuscript fluorescence has been replaced by florescence. P4L43 molecular technics detect the presence of AFBs DNA and not the presence of AFB P6L41 add the reference used for the grading of smear P8L14 clarify on the use of the gold standard. it is not clear for me how each microscopy was compared to culture. were the authors using any positive in three culture tests? table 1 show an important number of smear positive culture negative results in each microscopy methods. what could explain that? same question for table 4 with very low specificity of microscopy? what was the contamination rate of the culture method in each of the three digestion groups? a flow chart will surely improve the understanding of the study design

REVIEWER	Leopold Gustave Lehman University of Douala, Cameroon
REVIEW RETURNED	25-Oct-2013

GENERAL COMMENTS	The topic is very interesting in the present context of high prevalence of TB. The improvement of diagnosis with novel technologies is a great challenge. The focus of the authors has been well adressed and the results are quite interesting. The strenght and limitations of the study have also been very well

	presented by the authors. In my opinion, the paper can be published if some minor errrors are corrected: ie. In all the text : "fluorescence" instead of "florescence" In keywords "microbiology" in capital letters The 8th line of results "at least" instead of "at list" and slides instead of slide. Some errors may still be present. The authors should just read carefully.

REVIEWER	J Metcalfe University of California, USA
REVIEW RETURNED	10-Dec-2013

GENERAL COMMENTS	Abstract: Please place concentration (%) for NaOH Comparative test accuracy data should be succinctly covered in the Results. Mycobacterial growth should be referred to rather than “number of AFBs” Introduction: Please provide a context/review of the literature for the research question around digestion times. Methods: Please note whether this was a referral center or whether patients were seen at this location. Were the technicians carrying out the study equally experienced with all tested smear methods? Adherence to STARD criteria (http://www.stard-statement.org/) should be noted. Discussion: Can the authors comment on the large proportion of patients (~46%) with a clinical diagnosis of TB who were treated in absence of microbiologic confirmation? Specificities reaching 69% for PiLED are concerning and lower than other investigators have reported. Reasons behind this should be justified and discussed in the Discussion. Contrary to popular belief, sensitivity and specificity do vary with prevalence (e.g., see Brenner Stat Med 1997) Significant copy editing is still needed (e.g., see last sentence of first paragraph of Results)

VERSION 1 – AUTHOR RESPONSE

Response to comments by Reviewer #1 (Yap Boum II):

1. The paper written by Gambo et al evaluating different microscopy methods and digestion time for culture is quite relevant for RLS since the recommendation of WHO to rule out Fluorescence microscopy for TB diagnosis. However no ethical approval has been mentioned in the manuscript which is critical for the running of any study involving human subject

We thank the reviewer for this observation. We agree with the reviewer that ethical approval was not mentioned although we indicated under “study population” in the method section that these data were completely de-identified therefore eliminating potential risk if any to the patients that provided the samples studied. For clarity, we revised the statement as follows: “Ethical review was waived because in the opinion of the study center review committee there was no potential risk to participants’ safety,

privacy or confidentiality since there was no formal contact between investigators and participants either directly (interview, questionnaires, etc.) or indirectly (medical records, personal identifiers etc.). The sputum specimens provided for routine clinical care services were completely anonymized before they were analyzed for the study and there was no risk that the pooled samples can be de-anonymized through data linkages"

2. In the entire manuscript fluorescence has been replaced by florescence.
P4L43 molecular technics detect the presence of AFBs DNA and not the presence of AFB.

We appreciate the reviewer's attention to details and are thankful for these observations. Florescence has been replaced with fluorescence in the entire manuscript while AFB was deleted and DNA added on page 4 line 43 as correctly identified by the reviewer.

3. P6L41 add the reference used for the grading of smear
The reference was added as recommended.

4. P8L14 clarify on the use of the gold standard. It is not clear for me how each microscopy was compared to culture. Were the authors using any positive in three culture tests?

The comparison was made between the positive and negative outcomes of each microscopy to the similar outcomes in each of the three culture tests. The outcomes of each microscopy test were compared against the final outcomes of each of the three culture tests in the traditional decision matrix (Table 2), then the true positives, true negatives, false positives and false negatives deduced from table 2 were compared using matched sample frequency cells in Table 3 to demonstrate the degree of agreement and discordance between each of the microscopy test and the gold standards.

5. Table 1 show an important number of smear positive culture negative results in each microscopy methods. What could explain that?

We thank the reviewer for this comment. The frequencies of false positive microscopy tests were even higher for the fluorescence techniques. We believe some of the patients seen at this center may have chest infections other than TB with similar symptoms as TB, example a fungal pneumonia, nocardia, etc. which could be partially acid-positive. Some fluorescent particles present in the sputum may appear AFB positive due to low-power objective of the fluorescence techniques.

6. Same question for table 4 with very low specificity of microscopy?

The same explanation above applies to this question too.

7. What was the contamination rate of the culture method in each of the three digestion groups?

The contamination rates in the three digestion groups were as follows: 6% (10 min), 4% (15 min) and 7% (20 min).

8. A flow chart will surely improve the understanding of the study design

We thank the reviewer for this comment. A brief flow chart summarizing the study design is provided in Figure 1 below and in the revised manuscript.

Figure 1. Flow chart for the study design involving 450 sputum samples from 150 patients with clinical TB

Response to comments by reviewer #2 (Leopold Gustave Lehman)

1. The topic is very interesting in the present context of high prevalence of TB. The improvement of diagnosis with novel technologies is a great challenge. The focus of the authors has been well addressed and the results are quite interesting.

We appreciate the reviewer's complementary remarks.

2. The strength and limitations of the study have also been very well presented by the authors.

We thank the reviewer for this comment.

3. In my opinion, the paper can be published if some minor errrors are corrected: ie.
In all the text : "fluorescence" instead of "florescence"In keywords "microbiology" in capital letters

The reviewer's observations were noted and corrected

4. The 8th line of results "at least" instead of "at list" and slides instead of slide. Some errors may still be present. The authors should just read carefully.

We thank the reviewer for bringing our attention to these errors. They were located and corrected

Response to Reviewer #3 (J Metcalfe).

1.Please place concentration (%) for NaOH
We thank the reviewer for this comment. The concentration of NaOH and other reagents involved in the digestion process were indicated as follows: Mycoprep (4%NaOH-1%NLAC and 2.9% sodium

citrate)

2. Comparative test accuracy data should be succinctly covered in the Results.

We agree with the reviewer, the third paragraph in the results section was expanded to provide addition details on the comparative test accuracy data presented in Table 2.

3. Mycobacterial growth should be referred to rather than “number of AFBs”

We thank the reviewer for this observation, the recommended correction was effected.

Introduction: Please provide a context/review of the literature for the research question around digestion times.

We appreciate the reviewer’s feedback. Additional works by Krasnow, Allen and Abe were cited in the concluding part of the introduction section which mainly evaluated digestion methods and to a lesser extent digestion times.

Methods:

4. Please note whether this was a referral center or whether patients were seen at this location.

This center serves as the main tuberculosis treatment center in the northern Nigerian region. It is both a referral and a treatment center for fresh cases of TB

5. Were the technicians carrying out the study equally experienced with all tested smear methods?

We thank the reviewer for this comment. This was partly addressed in the STARD criteria checklist we provided earlier. The technicians were experienced with all tested smear methods although their general laboratory expertise varies; they were trained and retrained at various times on smear microscopy (covering Zeihl Neelsen and Fluorescence microscopy), Good Laboratory Practices and HIV rapid testing. They were equally trained on sputum smear panel slides preparation and are involved in panels preparation for Proficiency Testing (and supervision) in the country until present

6. Adherence to STARD criteria (http://www.stard-statement.org/) should be noted.

We appreciate the reviewers comment. STARD criteria noted and relevant modifications in the manuscript effected.

Discussion:

7. Can the authors comment on the large proportion of patients (~46%) with a clinical diagnosis of TB who were treated in absence of microbiologic confirmation?

We appreciate the reviewer’s concern. However, microbiologic confirmation is not part of the standard

of care at this center. Suspected cases of TB are treated according to the WHO recommended protocol for the DOT and STOP TB strategy programs which are based on positive smear results or strong clinical suspicion in smear negative cases.

8. Specificities reaching 69% for PiLED are concerning and lower than other investigators have reported. Reasons behind this should be justified and discussed in the Discussion.

We agree with the reviewer that the specificity for PiLED is lower than expected. Significant proportion of the patients may have co-infection with HIV which was believed to account for low specificity of LED-FM microscopy compared to the conventional ZN microscopy in two previous studies in settings with high burden of the disease (Chaidir Plos One 2013 and Cattamanchi Int J Tuberc Lung Dis 2009) although none of the reported low specificities was in the 70% range as in our case. Some fluorescent particles present in the sputum may appear AFB positive due to low-power objective of the fluorescence techniques which could account for the high false positive rate seen with the PiLED microscopy.

9. Contrary to popular belief, sensitivity and specificity do vary with prevalence (e.g., see Brenner Stat Med 1997).

We appreciate this observation and the reviewer's generosity for the citation provided.

10. Significant copy editing is still needed (e.g., see last sentence of first paragraph of Results).

The reviewer is absolutely right. These errors have been corrected and we are grateful for this important observation.

Again, we thank you and the reviewers for their sacrifice, timely review and highly constructive criticisms that have added strength and quality to our piece of work. We hope that we have satisfactorily addressed the reviewers' comments and that our manuscript will be considered for publication in the prestigious British Medical Journal (BMJ) Open.

VERSION 2 – REVIEW

REVIEWER	Yap Boum II Epicentre Uganda Research Centre
REVIEW RETURNED	19-Jan-2014

GENERAL COMMENTS	very interesting comparison of microscopies methods and decontamination time. in L7 page 3 syill florescence instead of fluorescence add the p value in page 10 with the results to show what is significant The researchercher MUST discuss the high proportion of microscopy positive (AFB positive) for samples that are culture Negative. they have assessed the time for decontamination but this may show that the concentration of NaOH is too high... no smear positive should culture negative especially as the culture is the gold standard... they should also mention the contamination rate by time of exposure as this is an important indicator of the decontamination process

VERSION 2 – AUTHOR RESPONSE

a) Very interesting comparison of microscopies methods and decontamination time.
in L7 page 3 still florescence instead of fluorescence

We thank the reviewer for this comment. The correct spelling of fluorescence is reflected in line 7 of page 3 of the manuscript as suggested by the reviewer.

b) Add the p value in page 10 with the results to show what is significant.

We appreciate the reviewer's comment. However, the results in page 10 were intended to provide the reader with the descriptive statistics summarized in Table 1. There was no comparison involved and as such no degree (e.g. p-values) or measure (e.g. odds ratio) of associations were provided here. The p-values were however added in Table 4 and in the text that preceded the table in which the different smear techniques were compared.

c) The researcher MUST discuss the high proportion of microscopy positive (AFB positive) for samples that are culture Negative.

Some of the reasons for the unusually high proportion of AFB positive, culture negative specimen could possibly be that some of the patients contrary to their claim have actually been on TB treatment at presentation. This may have adversely hindered the bacilli ability to grow, or more importantly in this case, the bacilli may have been killed by the excessive decontamination in the samples decontaminated for up to 20 minutes especially among cases with paucibacillary disease due to HIV co-infection. These patients may likely test positive to both smear microscopy and culture in repeat examinations with optimal decontamination time. Fungal infections are also not uncommon in the study area and together with some artefacts may have added to the high frequency of the smear positive, culture negative findings

d) they have assessed the time for decontamination but this may show that the concentration of NaOH is too high... no smear positive should culture negative especially as the culture is the gold standard... they should also mention the contamination rate by time of exposure as this is an important indicator of the decontamination process

We thank the reviewer for this comment. The contamination rate decreases with increasing exposure and perhaps the above 1% concentration of NaOH used contributed significantly in eliminating the contaminating bacteria as well as the few mycobacteria present especially among subjects with scanty AFB positive smears.

Again, we thank you and the reviewers for their sacrifice, timely review and highly constructive feedbacks that have added strength and quality to this manuscript. We hope that we have satisfactorily addressed the reviewers' comments and that our manuscript will be considered for publication in the prestigious British Medical Journal (BMJ) Open.